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Isolation of multiple nontuberculous mycobacteria species in the same patients

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SUMMARY

Background: During the observation of many patients with nontuberculous mycobacteria (NTM) infections, we have often isolated different NTM species in the same patient. In this study we elucidated the patterns of multiple NTM species isolation.
Methods: The analysis included all patients from whom more than one species of mycobacteria were cultured from a respiratory specimen at Seoul National University Hospital, a tertiary referral hospital in South Korea, between January 2002 and December 2008. The demographic characteristics and clinical and radiographic findings were reviewed retrospectively.
Results: Multiple NTM species were isolated from 37 patients. Four patients with *Mycobacterium gordonae*, *Mycobacterium terrae*, or *Mycobacterium lentiflavum* were excluded due to the possibility of contamination. Transition to another NTM species was observed in 23 patients (69.7%), while alternating isolation of two or three NTM species was seen in nine patients. Two species were isolated simultaneously from different sputum samples collected from one patient on the same day.
Conclusions: Different NTM species were isolated from the same patients.

1. Introduction

Nontuberculous mycobacteria (NTM) are ubiquitous organisms, which can be isolated from many environments,[1,2] and more than 125 NTM species have been identified.[3,4] Although the majority of NTM species are considered opportunistic pathogens, some NTM, such as *Mycobacterium avium-intracellulare*, *Mycobacterium kansasii*, and *Mycobacterium abscessus*, can cause disease in immunocompetent hosts.[2,5]

The treatment of NTM disease is challenging not only because of the long treatment period with multiple antimycobacterial drugs, but also the various treatment responses. Generally, selecting a regimen for treating NTM disease has been based on NTM species identification rather than in vitro drug susceptibility tests, because the clinical response does not correlate well with the in vitro susceptibility to many drugs used to treat NTM disease.[2]

It is possible to isolate different NTM species from the same patient during observation or treatment. In fact, the co-existence of *M. avium-intracellulare* was observed in 8% of 154 patients with lung disease caused by rapidly growing mycobacteria.[6] While observing many patients with NTM,[7] we also noted that different kinds of NTM species could be isolated in the same patient. In this study we elucidated the patterns of multiple NTM species isolation.

2. Methods

2.1. Study subjects

The analysis included all patients from whom different mycobacterial species were cultured from respiratory specimens at Seoul National University Hospital, a tertiary referral hospital in South Korea, between January 2002 and December 2008. The study protocol was approved by the institutional review board of Seoul National University Hospital.

2.2. Specimen processing

Respiratory specimens such as sputum were decontaminated with 4% NaOH, homogenized, and concentrated by centrifugation at 3000 *g* for 20 min. Processed sediment or a specimen from a normally sterile site was stained using the Ziehl–Neelsen method.[2] The acid-fast bacillus (AFB) smear results were graded according to the American Thoracic Society (ATS)/Centers for Disease Control and Prevention (CDC) classification.[8] Concentrated specimens were cultured in 3% Ogawa medium and observed weekly for

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9 weeks after inoculation. Once cultured, *Mycobacterium tuberculosis* and NTM were distinguished using the Gen-Probe method (Gen-Probe®, Gen-Probe, San Diego, CA, USA).[9] A polymerase chain reaction–restriction fragment length polymorphism method was used if the clinicians decided to identify the NTM to the species level.[10]

2.3. Data collection and review

The demographic characteristics, clinical findings, and laboratory results were obtained from a retrospective review of the medical records. The chest radiographs were evaluated by a board-certified radiologist (Dr C.M. Park), who was blinded to the patients' clinical records. The radiographic characteristics were evaluated based on a simple chest radiograph and computerized tomograms, if available.

3. Results

3.1. Number and characteristics of the patients with multiple NTM species

One thousand eight hundred and one patients with NTM isolates were identified for the analysis. From these, 4316 NTM were isolated during the study period. In 793 isolates from 532 patients, clinicians recognized the likelihood that the isolate was a pathogen rather than a colonizer and species identification was requested. Mycobacterial species identification was performed two or more times for 133 patients. Finally, 37 of 133 patients were confirmed as having multiple species of NTM. Among these, we omitted two patients with *Mycobacterium gordonae*, one patient with *Mycobacterium terrae*, and one patient with *Mycobacterium lentiflavum*, which are known to contaminate clinical specimens very easily.[2]

The median age of the patients was 60 years and 15 (45.5%) were male. Seventeen (51.5%) of the patients had a history of pulmonary tuberculosis and five (15.2%) had diabetes. Sputum (13 patients, 39.4%) and cough (13 patients, 39.4%) were their most frequent symptoms. After isolating the first NTM, these patients were followed for a median of 47.2 months (range 6.7–84.0 months). When the second NTM species was isolated, six patients (18.2%) had completed their treatment, seven (21.2%) were being treated, and 20 (60.6%) were being observed without treatment (Table 1).

Table 1
Characteristics of the 33 patients with multiple species of NTM

Number of patients	33
Age, years, median (range)	60 (25–79)
Male	15 (45.5%)
Ever-smoker	5 (15.2%)
Body mass index (kg/m^2)	21.0 ± 2.7
History of pulmonary tuberculosis	17 (51.5%)
Remote (more than 5 years ago)	11
Recent (within 5 years)	6
Comorbidities	
Chronic obstructive lung disease	2 (6.1%)
Bronchiectasis	14 (42.4%)
Diabetes	5 (15.2%)
Malignancy	6 (18.2%)
Connective tissue diseases	2 (6.1%)
Chronic kidney disease	1 (3.0%)
Immunosuppressant therapy	1 (3.0%)
Symptoms	
Asymptomatic	5 (15.2%)
Cough	13 (39.4%)
Sputum	13 (39.4%)
Dyspnea	1 (3.0%)
Hemoptysis	14 (42.4%)
Chest pain	1 (3.0%)
Weight loss	9 (27.3%)
Fatigue	3 (9.1%)
Laboratory findings	
Leukocytes (× 10^9/l)	7.113 ± 2.940
Hemoglobin (g/dl)	13.3 ± 1.3
Cholesterol (mg/dl)	178.9 ± 35.3
Albumin (g/dl)	3.96 ± 0.45
Follow-up duration after initial isolation of NTM, months, median (range)	47.2 (6.7–84.0)
Treatment state on isolation of second species of NTM	
Complete treatment	6 (18.2%)
Being treated	7 (21.2%)
Being observed without treatment	20 (60.6%)

NTM, nontuberculous mycobacteria.

3.2. Patterns of multiple NTM isolation

Among the 33 patients included in this study, two NTM species were isolated from 29 patients (87.9%), and three species were isolated from four patients. All but one patient were diagnosed as having lung diseases caused by the initially isolated NTM, based on the criteria suggested by the ATS in 2007.[2] One patient with simultaneous isolation of *M. avium* and *M. abscessus* did not satisfy the ATS criteria.

For 23 patients (69.7%), the isolated NTM changed to a different species, most frequently from *M. avium* to *M. abscessus* (six patients). Changes from *M. avium* to *M. intracellulare* (three patients) and from *M. abscessus* to *M. avium* (three patients) were also observed. Alternating isolation of two or three NTM was also observed in nine patients (27.3%), such as from *M. abscessus* to *M. avium* to *M. abscessus*. In addition, we simultaneously isolated two species from different sputum samples collected from one patient on the same day (Table 2).

3.3. Radiographic findings of the patients with multiple NTM species

Bronchiectasis (29 patients, 87.9%), bronchiolitis (29 patients, 87.9%), and nodules (28 patients, 84.8%) were the most common radiographic features among the 33 patients with multiple NTM species. A volume decrease (16 patients, 48.5%) and a cavity (10 patients, 30.3%) were also common. Involvement of the upper lobes and right middle lobe/lingular segment was more frequent, although lower lobe lesions were not rare (Table 3).

4. Discussion

We found that different NTM species could be isolated in the same patients and that there were transitional, alternating, and simultaneous patterns of change.

Although no studies have systematically analyzed the isolation of different NTM species, the co-existence of *M. avium-intracellulare* with rapidly growing mycobacteria has been recognized.[6] In addition, recent studies have identified the presence of different *M. avium-intracellulare* strains in the same patient.[11,12] In those studies, different strains were isolated from patients with nodular bronchiectatic features rather than cavitary disease.[11,12] Similarly, in our study, nodules (84.8%) and bronchiectasis (87.9%) were much more frequent than a cavity (30.3%) among the 33 patients with multiple NTM species isolates, suggesting that it is possible to have different NTM species in different ectatic bronchi or nodule sites.

The clinical implications of the isolation of different NTM species from the same patient are obscure. Wallace et al. proposed that isolating different strains of *M. avium-intracellulare* was accompanied by disease associated with the new strains.[11,12] However, this seems unlikely for our study population given that one third of patients with multiple NTM species showed the alternating isolation of two or more NTM species. Several possibilities (change of pathogen, co-pathogen, pathogen/colonizer, and so on) could account for our observation, but are still far from clear. The clinical significance of the isolation of multiple